大豆イソフラボンの抗アルツハイマー病効果（βアミロイドたん白凝集抑制作用）の検討

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Soybean Isoflavones Inhibit Amyloid β-protein Self-Assembly

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ABSTRACT

Epidemiological evidences from retrospective and case-control studies has indicated that estrogen replacement therapy can decrease the risk of developing Alzheimer's disease. Soybean isoflavones have been proposed as phytoestrogens, because some of isoflavones were reported to exert a neuroprotective effect against β-amyloid protein (Aβ)-induced neurotoxicity. Recently, our experimental studies have demonstrated that some kinds of flavonoids and estrogens inhibited Aβ assembly and destabilized Aβ aggregates. To examine the effects of isoflavones on the assembly of the two predominant disease-related Aβ alloforms, $Aβ_{1–42}$ and $Aβ_{1–40}$, here we used thioflavin T fluorescence, electron microscopy, and photo-induced cross-linking of unmodified proteins (PICUP) followed by SDS-PAGE. Initial studies revealed that some kinds of isoflavones blocked Aβ fibril formation. Subsequent evaluation of the assembly stage specificity of the effect showed that isoflavones were able to inhibit pre-protofibrillar oligomerization. These data suggest that isoflavones would be worthy of consideration as a therapeutic agent for Alzheimer's disease. *Soy Protein Research, Japan* **13**, 175-181, 2010.

Key words : Alzheimer's disease, amyloid β-protein fibrils; isoflavone; oligomerization; photo-induced cross-linking of unmodified proteins (PICUP)

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アルツハイマー病は認知症の原因の第一位を占め，脳内ベータ・アミロイドたん白（Aβ）の凝集と沈着が病態の最上流にあるとされる（アミロイドカスケード仮説）．従来，脳アミロイドとして蓄積するAβ線維（fAβ）が神経毒性を発揮すると考えられていたが，最近は可溶性オリゴマーの毒性が注目されている．

大豆イソフラボンは女性ホルモンと構造が似ているため（Fig. 1），類女性ホルモン作用が注目されている．近年の疫学調査では，女性ホルモンがアルツハイマー病発症の危険率を低下させ，その発症を遅延させると報告された[1, 2]．細胞実験では，イソフラボンがAβによる神経毒性を軽減し神経保護的に働くことが報告され，アルツハイマー病の認知機能障害を予防する可能性が報告された[3, 4]．しかし，その詳細なメカニズムは明らかにされていない．

これまで我々は，フラボン骨格（Fig. 1）を有する複数のフラボノイドがAβ凝集作用を示すことを*in vitro*[5 ~ 7]，および*in vivo*[8]で報告してきた．さらに生体内分子である女性ホルモン（Fig. 1）がAβ凝集作用を示すことを報告し，その抗アミロイド効果に着目してきた[9, 10]．

本研究では大豆イソフラボンが，Aβ凝集過程に対して直接的な抑制作用を有するかを検討することを目的とした．

方　　法

5種類のイソフラボン（Isof-1，Isof-2，Isof-3，Isof-4，Isof-5）（Fig. 1）について，A$\beta_{1\text{–}42}$およびA$\beta_{1\text{–}40}$の凝集過程，すなわちfAβ形成過程，Aβオリゴマー形成過程に及ぼす影響を検討した．fAβ形成過程の解析には，我々が確立しているA$\beta_{1\text{–}42}$およびA$\beta_{1\text{–}40}$を生体条件下で凝集させる試験管内モデル[11, 12]を用いて，チオフラビンTを用いた分光蛍光定量法にて経時的に定量・比較し，電子顕微鏡による形態観察にて半定量的に解析した．Aβオリゴマー形成過程の解析には，photo-induced cross-linking of unmodified proteins (PICUP)，SDS-PAGEを用いた[13]．

（倫理面への配慮）問題なし．

結　　果

解析したイソフラボンの一部において，チオフラビンTを用いた分光蛍光定量法にて，fA$\beta_{1\text{–}42}$形成抑制（Fig. 2），およびfA$\beta_{1\text{–}40}$形成抑制（Fig. 3）が濃度依存性に観察された．電子顕微鏡による形態観察を行ってfAβ構造形成が抑制されていることを確認した（Fig. 4）．特に強い抑制作用を示したのはIsof-1, Isof-3であった．また，fAβ形成抑制をみとめたイソフラボンにおいて，PICUPによるA$\beta_{1\text{–}42}$オリゴマー形成抑制（Fig. 5a），およびA$\beta_{1\text{–}40}$オリゴマー形成抑制（Fig. 5b）が観察された．

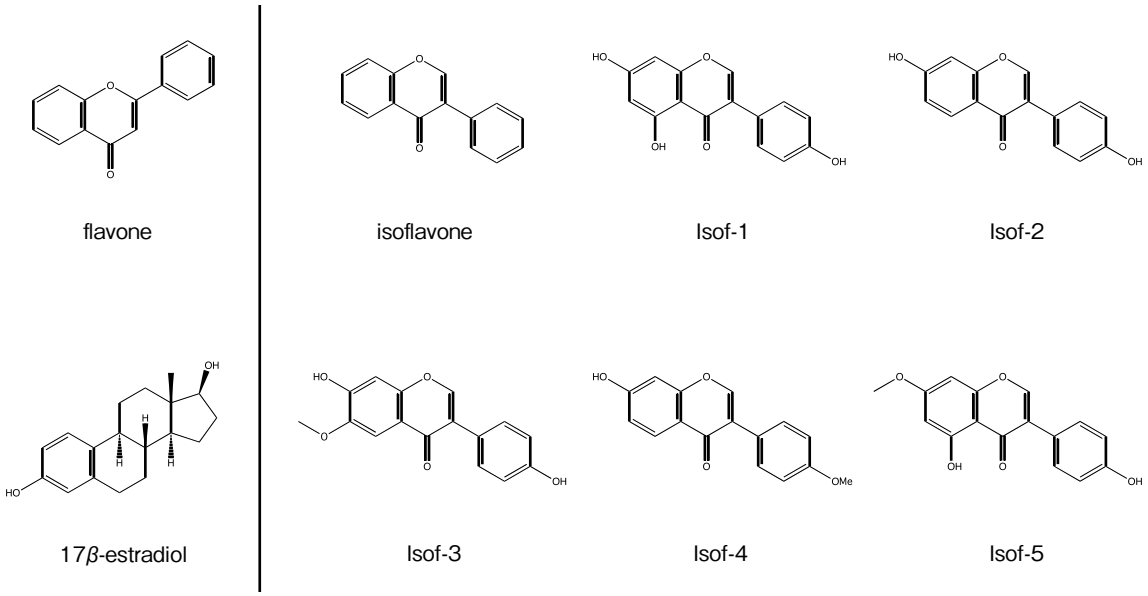

Fig. 1. Structures of isoflavones examined in this study and related molecules.

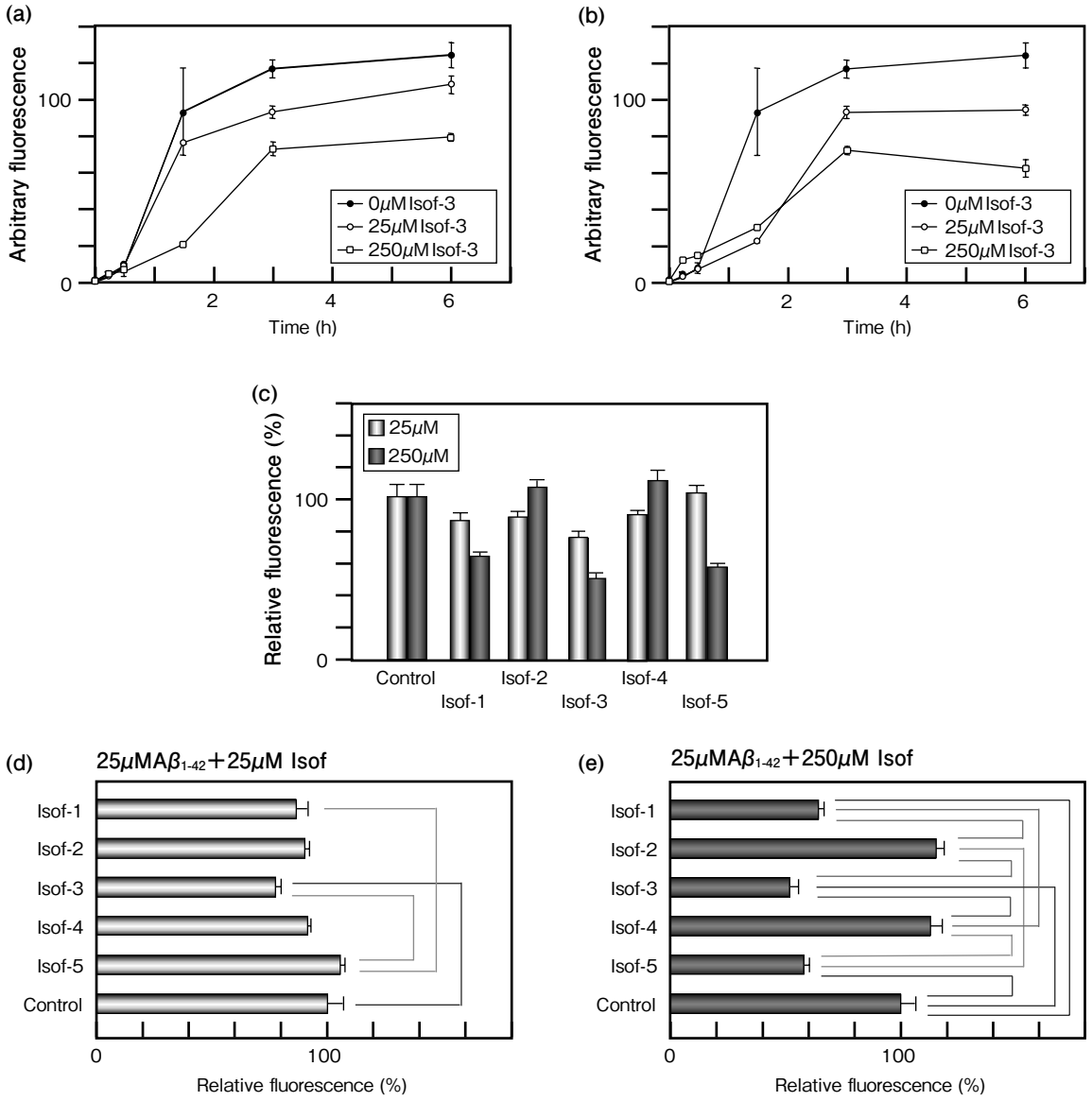

Fig. 2. ThT binding to $A\beta_{1-42}$ assembly. (a, b) Effects of Isof-1 (a) or Isof-3 (b) on the kinetics of $fA\beta_{1-42}$ formation from fresh $A\beta_{1-42}$. The reaction mixtures containing 25 μM $A\beta_{1-42}$, 10 mM phosphate buffer, pH 7.4, and 0 (closed circles), 25 (open circles), or 250 μM (open squares) of Isof-1 (a) or Isof-3 (b), were incubated at 37℃ for the indicated times. Periodically, three 5-μL aliquots were removed, and ThT binding levels were determined. Binding is expressed as mean fluorescence (in arbitrary fluorescence units) ± error bars (S.E.). Each figure comprises data obtained in 3 independent experiments. (c–e) Effects of isoflavones on the formation of $fA\beta_{1-42}$ from fresh $A\beta_{1-42}$. The reaction mixture containing 25 μM $A\beta_{1-42}$, 10 mM phosphate buffer, pH 7.4, and 25 (white columns) or 250 μM (gray columns) isoflavones was incubated at 37℃ for 24 h, respectively. Each column represents the average of 3 independent experiments. The average without compounds was regarded as 100%. S.E. is indicated by bars. $p<0.05$, post-hoc Tukey–Kramer tests.

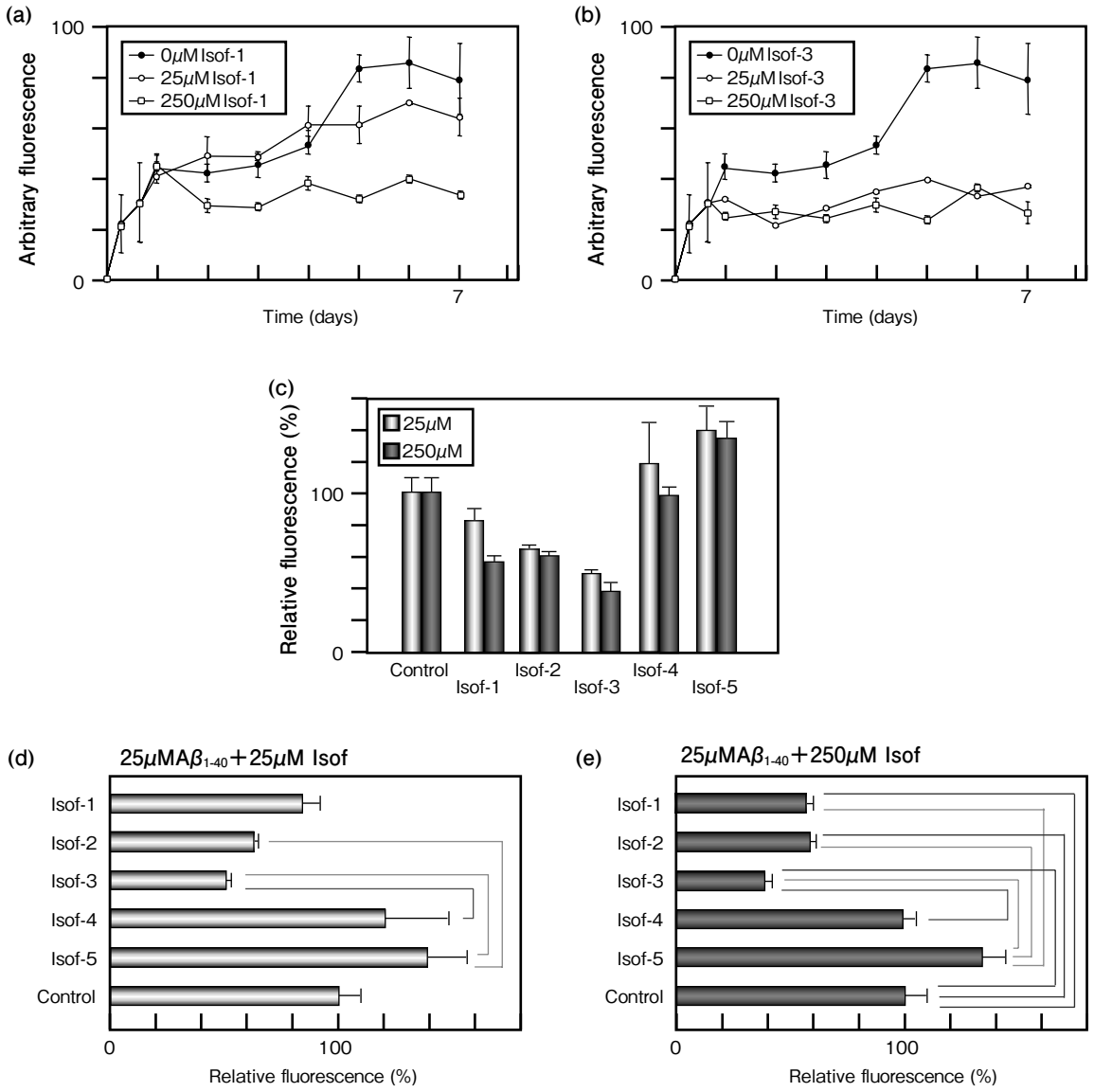

Fig. 3. ThT binding to Aβ_{1-40} assembly. (a, b) Effects of Isof-1 (a) or Isof-3 (b) on the kinetics of fAβ_{1-40} formation from fresh Aβ_{1-40}. The reaction mixtures containing 25 μM Aβ_{1-40}, 10 mM phosphate buffer, pH 7.4, and 0 (closed circles), 25 (open circles), or 250 μM (open squares) of Isof-1 (a) or Isof-3 (b), were incubated at 37℃ for the indicated times. Periodically, three 5-μL aliquots were removed, and ThT binding levels were determined. Binding is expressed as mean fluorescence ±S.E. Each figure comprises data obtained in 3 independent experiments. (c–e) Effects of isoflavones on the formation of fAβ_{1-40} from fresh Aβ_{1-40}. The reaction mixture containing 25 μM Aβ_{1-40}, 10 mM phosphate buffer, pH 7.5, and 25 (white columns) or 250 μM (gray columns) isoflavones was incubated at 37℃ for 7 days, respectively. Each column represents the average of 3 independent experiments. The average without compounds was regarded as 100%. S.E. is indicated by bars. $p<0.05$, post-hoc Tukey–Kramer tests.

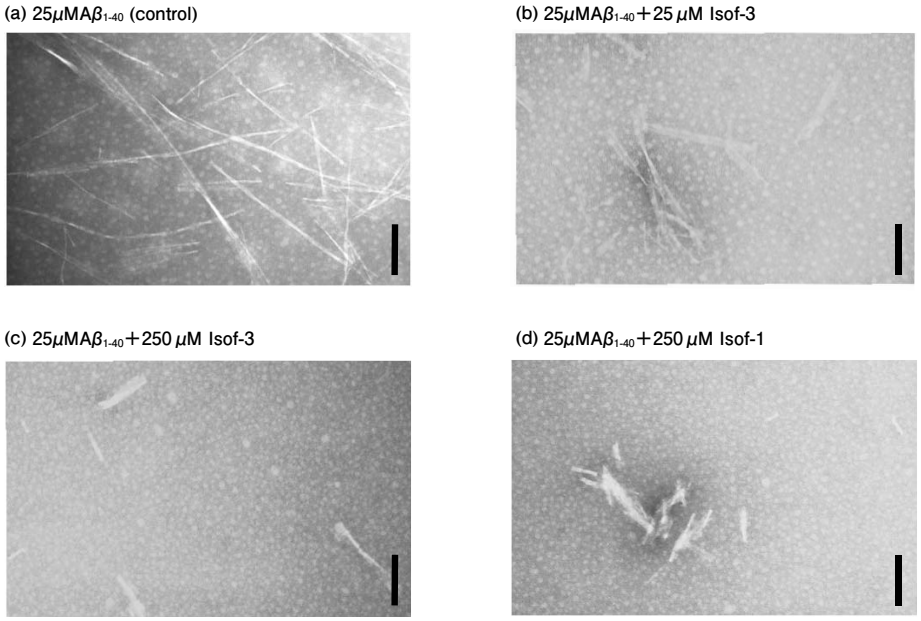

Fig. 4. Aβ assembly morphology. Electron micrographs were used to determine the morphologies of assemblies of Aβ_{1-40}. The reaction mixtures containing 25 μM Aβ_{1-40}, 10 mM phosphate buffer, pH 7.4, and 0 (a), 25 (b) or 250 μM Isof-3 (c), or 250 μM Isof-1 (d) were incubated at 37℃ for 0 (a), or 6 h (b, c, d). Scale bars indicate 250 nm.

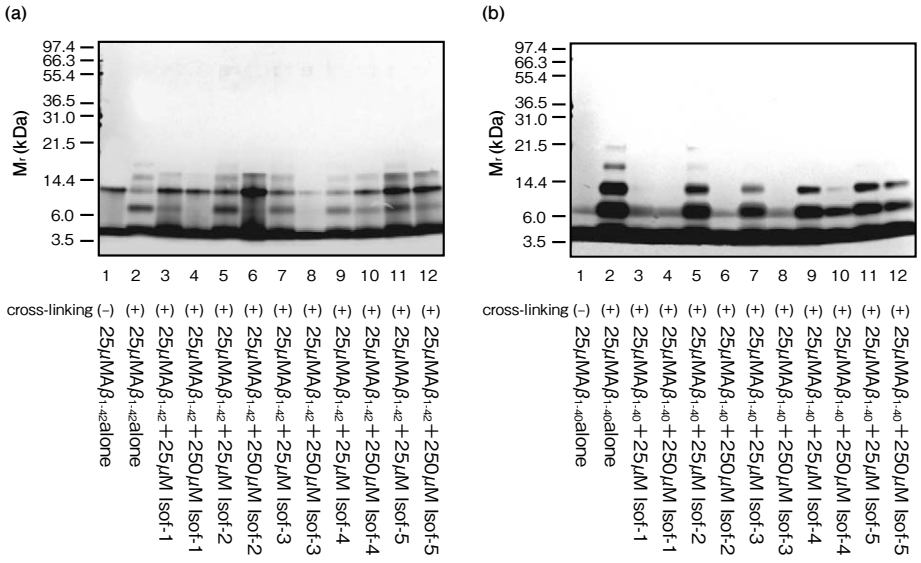

Fig. 5. Aβ oligomerization. PICUP, followed by SDS-PAGE and silver staining, was used to determine the effects of isoflavones on oligomerization of Aβ_{1-42} (a), or Aβ_{1-40} (b). Lanes 1, proteins alone (no cross-linking); lanes 2, proteins alone; lanes 3, proteins plus Isof-1 (25 μM); lanes 4, proteins plus Isof-1 (250 μM); lanes 5, proteins plus Isof-2 (25 μM); lanes 6, proteins plus Isof-2 (250 μM); lanes 7, proteins plus Isof-3 (25 μM); lanes 8, proteins plus Isof-3 (250 μM); lanes 9, proteins plus Isof-4 (25 μM); lanes 10, proteins plus Isof-4 (250 μM); lanes 11, protein plus Isof-5 (25 μM); and lanes 12, protein plus Isof-5 (250 μM). Each gel is representative of each of 3 independent experiments.

考　　察

本研究で解析したイソフラボンの一部が濃度依存性にfAβ_{1-42}，およびfAβ_{1-40}形成抑制作用を有することを見出した．またfAβ形成のみならず，凝集の早期段階，すなわちオリゴマー形成においても抑制作用を及ぼすが明らかになった．既に報告されているフラボノイドだけでなく，一部のイソフラボンも抗Aβ凝集作用を有していると考えられた．

これまで，イソフラボンがAβよる細胞毒性を軽減する神経保護作用の機序としては，estrogen receptor-mediated pathwayを介したり，抗酸化作用によるものなどと考察されてきた[4]．本研究ではAβ凝集そのものを抑制し，オリゴマー形成や線維形成を抑えることで，神経保護作用を示す可能性が考えられた．さらに，それぞれのAβ凝集体について，原子間力顕微鏡を用いた詳細な形態観察や，神経保護作用の有無の検討が必要である．

要　　約

大豆イソフラボンはアルツハイマー病の予防薬・治療薬開発の鍵となる可能性がある．

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